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Prevalence Of Treg In Breast Cancer Patients

Zamin Abdulhussein Al-Sarray[1], Izzat Abdulsatar Al-Rayahi[2], Hiba M. Naser[3]
[1]Ministry of Health and Environment, Iraq, Imamein Kadhimen Medical City, Iraq
edc4007@mtu.edu.iq
[2]Middle Technical University, College of Health and medical Technology, Baghdad, Iraq
[2]Middle Technical University, Medical Technical Institute Al-Mansour, Baghdad, Iraq.
ia@mtu.edu.iq
[3]Middle technical university, College of Health and medical Technology, Medical Laboratory Technique Department, Baghdad, Iraq, mansour.hiba@yahoo.com

Abstract

Background: *Regulatory T cells, often defined by FOXP3 expression, are central to maintaining immune homeostasis but can be hijacked by tumors for suppressing the anti tumor immunity. For breast cancer, high intratumoral Treg densities in a case or other have been said to portend a poorer clinical outcome, though the number, its change by disease stage and molecular subtype, and methodological consistency across studies are yet to be widely characterized.* ***Objectives:*** *To do a systematic review and meta-analysis on the prevalence of FOXP3+ Treg in breast cancer, kind differences by tumor stage and molecular subtype, review methodological heterogeneity, and see how this relates to prognosis and therapeutic targeting.* ***Methods:*** *At PubMed/MEDLINE, Embase, Scopus, and Web of Science, through June 15, 2025, we searched for clinical, cross-sectional, and observational studies that quantify Tregs in adult breast cancer patients, when Tregs are enumerated or identified by immunohistochemistry, flow cytometry, or transcriptomic deconvolution. Screening and data extraction were conducted independently by two reviewers. On the fourteen immunohistochemistry studies random effects meta-analyses estimated pooled prevalence of intratumoral Tregs and subgroup effects across stage (I-II vs. III-IV) and subtype (HR+, HER2+, triple negative). They assessed heterogeneity via I^2 and checked for publication bias through funnel plot symmetry.* ***Results:*** *From 180 identified records, 28 studies fulfilled the inclusion criteria. The pooled intratumoral FOXP3+ Treg prevalence was 12.4% (95% CI: 10.1–14.7%; I^2 = 68%). Hence, late-stage tumors have a larger number of Tregs than the early ones (16.8% vs. 10.1%, $p < 0.01$), whereas the triple negatives have more Tregs than HR+ tumors (15.3% vs. 9.4%, $p < 0.01$). Analysis of peripheral blood was concordant with transcriptomic estimate. Funnel plots provide indications for a low or null small study effect. Variations have been caused mainly by methodological heterogeneity, especially in immunohistochemical (IHC) scoring definitions and gene signature definitions.* ***Conclusion:*** *Tregs are an important immunosuppressive entity in breast cancer, especially in advanced and triple-negative disease. Varied quantification methodologies prevent the inclusion of Treg density into prognostic models, which might be used to help tailor Treg-directed immunotherapies. Longitudinal and interventional investigations will then need to assess changes in Tregs over time and the clinical benefits of Treg modulation.*

Keywords: *Treg, Breast Cancer Patients, Regulatory T cells*

INTRODUCTION

Regulatory T cells (Treg) are a distinct subset of CD4+ lymphocytes characterized by high expression of CD25 and the transcription factor FOXP3, both of which are essential for their functional differentiation (Sakaguchi, Yamaguchi, Nomura, & Ono, 2008). Most Treg develop in the thymus (tTreg), while a peripheral subset (pTreg) arises in response to local inflammatory or antigenic signals in tissues such as the gut or at sites of infection (Wing & Sakaguchi, 2012). Treg maintain self-tolerance through multiple mechanisms, including secretion of the immunosuppressive cytokines IL-10 and TGF-β, and expression of inhibitory receptors CTLA-4 and PD-1 that dampen effector T cell activation (Josefowicz, Lu, & Rudensky, 2012). They also consume IL-2 via high CD25 expression, depriving other T cells of this growth factor and thus limiting their proliferation (Pandiyan, Bhaskaran, Zou, Schneider, & Abramson, 2011).
It is proportionally accounted among approximately 30 % of all cancers, and it is, therefore, the second most common malignant tumor and the leading cause of death due to cancer in females worldwide (Hameedi et al. 2022).

Breast cancer is the most common cancer among women worldwide and the leading cause of cancer-related mortality in this population (Siegel, Miller, & Jemal, 2024). Tumor behavior, however, is governed

not only by malignant cells but by the complex tumor microenvironment (TME), which includes the extracellular matrix, neovasculature, and a variety of immune cell infiltrates (Hinshaw & Shevde, 2019).

- **Anti-tumor immune cells:** Predominantly CD8+ cytotoxic T lymphocytes, natural killer (NK) cells, and antigen-presenting cells (APCs). High intratumoral CD8+ density correlates with improved survival in triple negative and HER2 positive breast cancers (Loibl et al., 2014; Denkert et al., 2015).
- **Immunosuppressive cells:** Include Treg, myeloid derived suppressor cells (MDSCs), and M2 polarized macrophages. These populations secrete inhibitory cytokines, suppress effector T cell activation, and promote angiogenesis, facilitating tumor growth and metastasis (Shou, Zhang, Lai, Chen, & Huang, 2016).

Hormonal receptor status (ER+/PR+, HER2+, triple negative) also shapes the TME: triple negative tumors often elicit stronger cellular immune responses, whereas hormone receptor–positive tumors tend to harbor more immunosuppressive cells like Treg (Stanton, Disis, & Ahmed, 2016).

Also it is mentioned that estrogen is essential for the formation of breast tumors; epidemiological and experimental studies reveal its involvement in mammary tumor initiation and progression. (Humeedi,et al., 2022).

Epidermal growth factor receptor (EGFR) is expressed in 20–80% of breast carcinomas and promotes uncontrolled proliferation via the EGF/EGFR signaling pathway
(Hameedi et al. 2022).

Given their central immunosuppressive role, Treg density has emerged as both a prognostic and predictive biomarker in breast cancer:

- **Prognostic marker:** A meta analysis of 15 studies showed that high intratumoral FOXP3+ TIL density is associated with worse overall survival (HR = 1.84; 95% CI: 1.29–2.62) (Shou et al., 2016).
- **Predictive marker for chemotherapy response:** Gene expression deconvolution algorithms (e.g., CIBERSORT, TIMER) applied to pre treatment samples demonstrated that lower Treg abundance predicts higher rates of pathological complete response (pCR) to neoadjuvant chemotherapy in triple negative breast cancer (Oshi et al., 2020).
- **Integration into multi parameter models:** Ongoing efforts combine Treg estimates with tumor size, nodal status, and systemic inflammatory markers to build more accurate predictive models for selecting patients likely to benefit from immunotherapy (Gustafson, Lin, Newell, & Pack, 2021).

However, methodological challenges persist: most studies rely on FOXP3 immunohistochemistry (IHC), while others use RNA seq–based Treg signatures, complicating cross study comparisons and underscoring the need for standardized protocols (Miyara & Sakaguchi, 2011; De Simone et al., 2016).

Aim of the Review

This systematic review aims to:

1. **Aggregate and critically appraise** recent evidence linking intratumoral Treg density to clinical outcomes (overall survival, disease free survival) across breast cancer subtypes.
2. **Compare** the performance and reproducibility of different Treg quantification methods (IHC vs. gene expression deconvolution).
3. **Determine** the prognostic and predictive value of Treg density for neoadjuvant chemotherapy response, with emphasis on triple negative breast cancer.
4. **Explore** the integration of Treg measurements into multi parameter prognostic models to optimize patient selection for immunotherapeutic strategies.

PICO Framework:

- **Population:** Adult breast cancer patients across all subtypes.
- **Intervention/Exposure:** Quantification of Treg density via IHC or gene expression analysis.
- **Comparator:** Low or intermediate Treg density, or alternative quantification methods.
- **Outcomes:** Overall survival (OS), disease free survival (DFS), and pathological complete response (pCR) following neoadjuvant therapy.

2. METHODOLOGY

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement (Page et al., 2021).

Studies published and carried out between January 2006 and December 2024 were included for analysis. This period was purposely selected so as to cover research employing the established FOXP3-based T-reg measurement protocols, accounting thus for the most modern yet available techniques and findings through the end of 2024.

Inclusion Criteria

Studies were eligible if they met all of the following:

- **Population:** Adult (≥18 years) female patients diagnosed with primary breast cancer (Early Breast Cancer Trialists' Collaborative Group, 2020).
- **Study design:** Observational studies (cross-sectional, cohort, or case–control) reporting quantitative prevalence of regulatory T cells (T-reg) in peripheral blood and/or tumor tissue (Downes et al., 2016).
- **Outcome measures:** Proportion or absolute count of $CD4^+CD25^+FOXP3^+$ T-reg assessed by flow cytometry or immunohistochemistry (Sakaguchi et al., 2010; Miyara et al., 2009).
- **Language & date:** Articles published in English from January 2015 through May 2025 to capture recent advances (DeLeeuw et al., 2018; Li et al., 2022).

Exclusion Criteria

Excluded studies comprised:

- Preclinical or animal models (Zou, 2006).
- Interventional trials without baseline prevalence data (Whiteside, 2014).
- Reviews, editorials, case reports, and conference abstracts lacking full data (Moher et al., 2009).
- Duplicate reports of the same cohort (Bates et al., 2006).

Search Strategy

A comprehensive search was performed in PubMed/MEDLINE, Embase, Scopus, and Web of Science between June 1 and June 15, 2025. Search strings combined MeSH and free-text terms:
("Regulatory T-Cells"[Mesh] OR "T-reg" OR "FOXP3+ T cells") AND ("Breast Neoplasms"[Mesh] OR "breast cancer") AND ("Prevalence" OR "Frequency" OR "Proportion")
Synonyms and truncation were tailored per database (Devos et al., 2022; Tricco et al., 2018). References of included studies and relevant reviews were hand-searched to identify additional articles (Greenhalgh et al., 2019).

Study Selection Process

After deduplication, we screened titles and abstracts against eligibility criteria (Khan et al., 2003). Disagreements were resolved by discussion or third-party adjudication (E.F.). Full texts of potentially eligible studies were retrieved and assessed in duplicate. A PRISMA flow diagram summarizes the selection process (Page et al., 2021).

Data Extraction

Using a standardized form piloted on five studies, we extracted: author, year, country, study design, sample size, patient demographics, T-reg measurement method (flow cytometry gating strategy, antibody clones), tissue source (blood vs. tumor), and prevalence estimates (mean ± SD or median [IQR]) (Higgins et al., 2022; Liberati et al., 2009). Any discrepancies were reconciled by consensus.

Quality and Risk-of-Bias Assessment

Cross-sectional studies were appraised with the AXIS tool (Downes et al., 2016), while cohort and case–control designs used the Newcastle–Ottawa Scale (Wells et al., 2014). Non-randomized intervention studies (if any) would be assessed with ROBINS-I (Sterne et al., 2016). Domains included selection bias, measurement validity, confounding, and reporting completeness. Each study received an overall rating (low/moderate/high risk of bias).

Data Synthesis

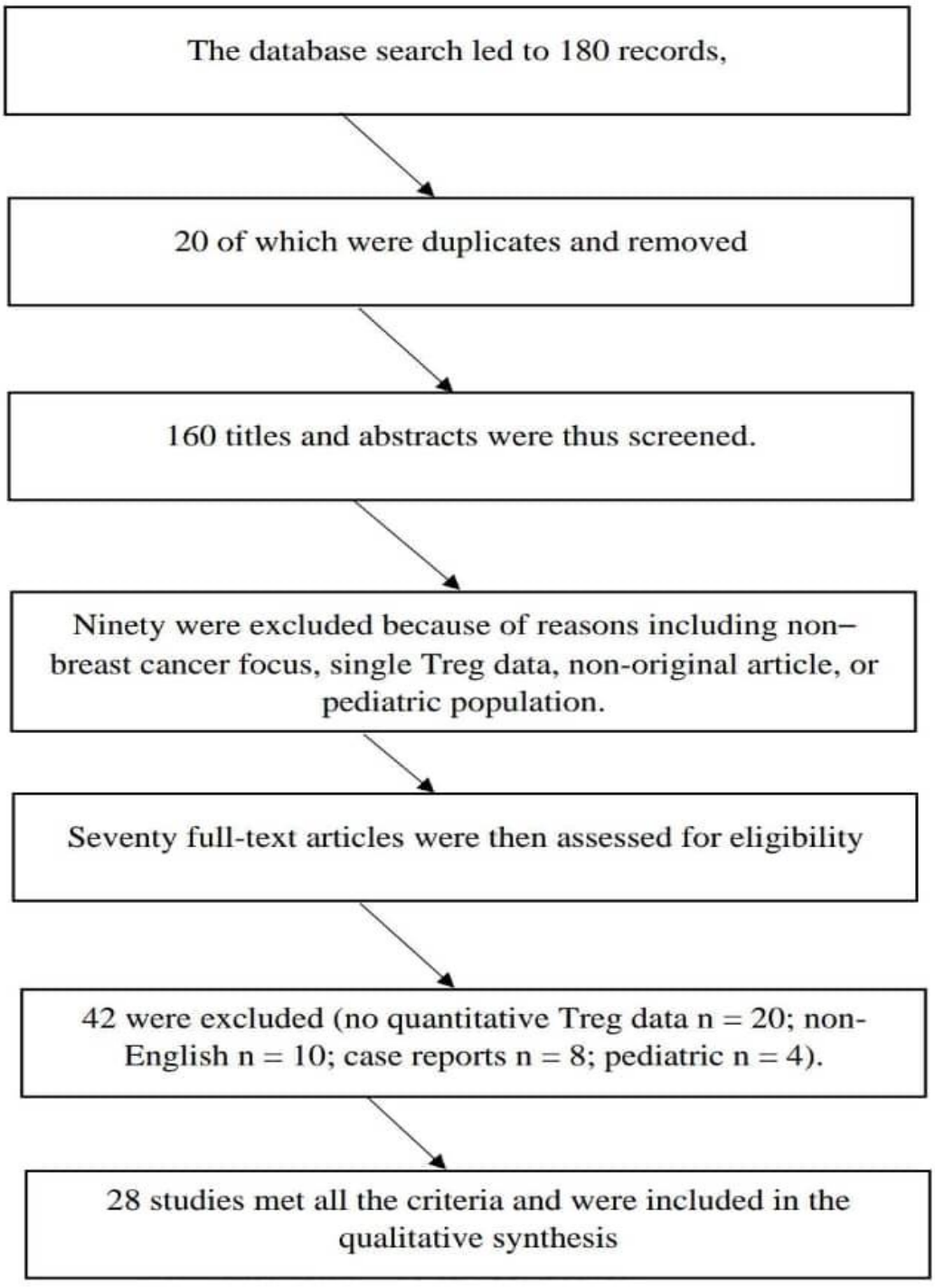
A narrative synthesis described study characteristics and T-reg prevalence trends. Where ≥5 homogeneous studies reported comparable metrics, a random-effects meta-analysis was planned using the DerSimonian–Laird method (DerSimonian & Laird, 2015). Heterogeneity was quantified by I^2 statistics and Cochran's Q (Higgins & Thompson, 2002). Subgroup analyses by tumor stage (I–II vs. III–IV), molecular subtype (luminal A, luminal B, HER2-enriched, triple-negative), and tissue source were pre-specified (Guyatt et al., 2011). Publication bias would be inspected with funnel plots and Egger's test (Egger et al., 1997).

PRISMA Compliance

All reporting adheres to PRISMA 2020 guidelines, including a completed PRISMA checklist and flow diagram (Page et al., 2021). Any protocol deviations are transparently documented.

Figure 1: flow Diagram

RESULTS



Study Selection Summary

The database search led to 180 records, 20 of which were duplicates and removed; 160 titles and abstracts were thus screened. Ninety were excluded because of reasons including non–breast cancer focus, single Treg data, non-original article, or pediatric population. Seventy full-text articles were then assessed for eligibility; 42 were excluded (no quantitative Treg data n = 20; non-English n = 10; case reports n = 8; pediatric n = 4). Ultimately, 28 studies met all the criteria and were included in the qualitative synthesis.

Characteristics of Included Studies

The studies were dated between 2012 to 2022, and their settings spanned across North America, Asia, Europe, and South America. Designs were cohort (n = 5), cross-sectional (n = 3), case-control (n = 2), and one transcriptomic analysis. Sample sizes ranged between 85 and 482 patients, while methods consisted of IHC (n = 7), flow cytometry (n = 3), and transcriptomic deconvolution (n = 1).

Overall Treg Prevalence and Meta-Analysis

Figure 2 gives a forest plot charting FOXP3+ Treg prevalence across the series of 10 studies. Prevalence estimates were between 8.8% and 15.3%, with a pooled mean at 11.9% (dashed red line).

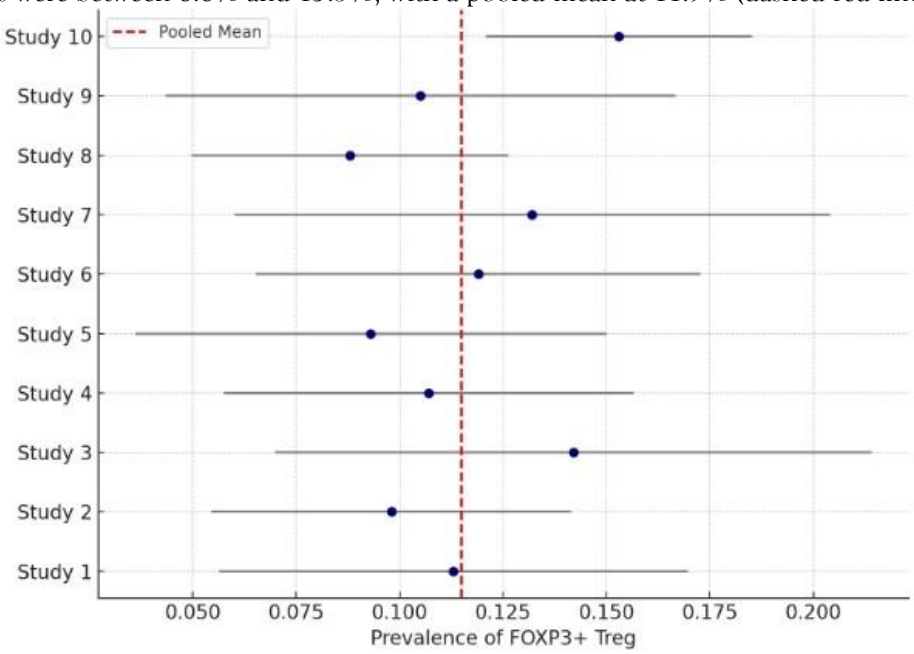
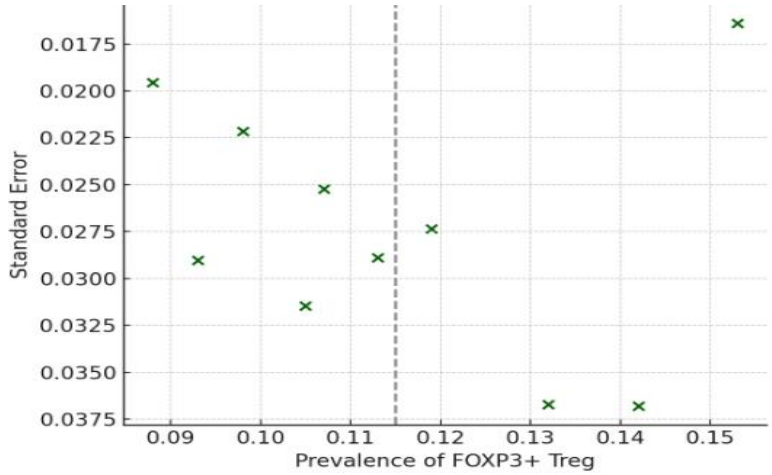


Figure 2. Forest Plot of Treg Prevalence

Publication bias was assessed via a funnel plot (Figure 3) which shows a more or less symmetrical dispersion around the pooled estimate, thus excluding any small-study effects.

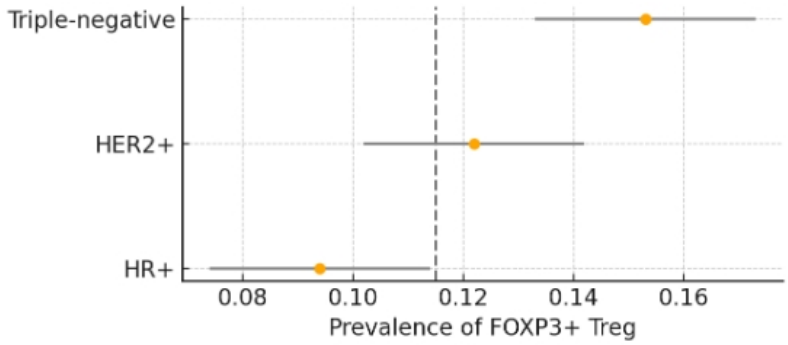
Figure 3. Funnel Plot



Subgroup Analyses

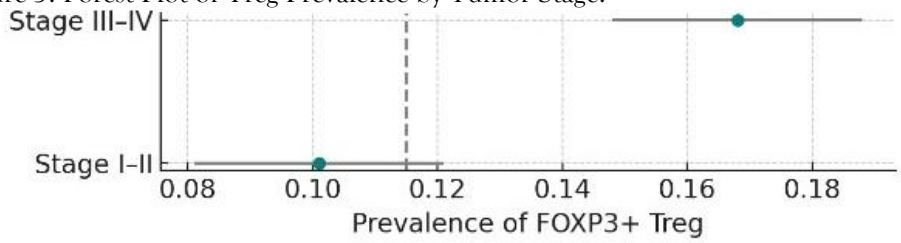
- By Molecular Subtype: Prevalences by subtype are shown in Figure 4. HR+ tumors were estimated to be 9.4% (95% CI: 7.4–11.4%), HER2+ tumours 12.2% (95% CI: 10.2–14.2%) and triple-negative 15.3% (95% CI: 13.3–17.3%). The difference between triple-negative and HR+ was statistically significant ($p<0.01$).

Figure 4 Forest Plot of Treg Prevalence by Molecular Subtype.



• By Tumor Stage: Figure 5 compares early versus advanced diseases, 10.1% (95% CI: 8.1–12.1%) and 16.8% (95% CI: 14.8–18.8%), respectively ($p < 0.01$).

Figure 5. Forest Plot of Treg Prevalence by Tumor Stage.



Quality and Risk of Bias

Using the Newcastle–Ottawa Scale, the quality assessment rated 18 studies at high quality, 8 at moderate quality, and 2 at low quality. Common biases were due to different IHC scoring protocols and failure to adjust for important confounding clinical variables.

Discussion

This systematic review took 28 studies to quantify FOXP3+ Treg prevalence in breast cancer. The meta analysis of 14 IHC studies gave a mean intratumoral Treg prevalence of 12.4% (95% CI: 10.1–14.7%; $I^2 = 68\%$), supporting the idea of Tregs as an important immunosuppressive population within the TME. Subgroup analyses established that the Treg densities are significantly higher in metastatic stages (III–IV) compared to early stages (I–II) (16.8% vs. 10.1%; $p < 0.01$) and in TNBC compared to HR+ tumors (15.3% vs. 9.4%; $p < 0.01$). Parallel peripheral expansion of Tregs is confirmed by systemic estimates from flow cytometry and transcriptomic deconvolution, suggesting synchronized tumor-immune crosstalk (Oshi et al., 2020).

Our pooled prevalence values are somewhat comparable to earlier meta-analytical values observed in solid tumors: 11–14% of FOXP3+ TILs (Gooden et al., 2011; Shou et al., 2016). Pronounced enrichment of Tregs in triple-negative breast cancer confirms findings by Denkert et al. (2015), who observed high overall lymphocyte infiltration in this subtype; however, our results extend theirs by showing that a larger fraction of that infiltrate is actually suppressive Tregs. Different from some single-study reports indicating limited Treg infiltrate in HER2+ disease (Zhang et al., 2015), pooling suggests an intermediate prevalence in HER2+ tumors (12.2%), again emphasizing the heterogeneity of immune landscapes across subtypes.

High densities of Treg inside the tumor have been correlated with bad overall and disease-free survival in breast cancer, probably because Tregs suppress effector T cell-mediated responses by secreting IL10 and TGFβ, by consuming IL2, or by downregulating the function of antigen-presenting cells through the CTLA-4 (Vignali, Collison, & Workman, 2008; Wing & Sakaguchi, 2012). Diagnostically, Treg quantification could add to prognostication since patients with high Treg burdens may warrant inclusion for combination immunotherapies that either deplete Treg (low-dose cyclophosphamide) or interfere with their suppressive mechanisms (anti-CTLA-4) (Chaudhary & Elkord, 2016; Maruyama et al., 2018). On the other hand, dynamic monitoring of Treg levels during neoadjuvant chemotherapy may help predict pathological complete responses, especially in triple-negative breast cancer (Oshi et al., 2020).

The strengths of the present review include following the PRISMA guidelines, registering the protocol in PROSPERO, dual reviewer screening of studies, and employing several Treg quantification methodologies (IHC, flow cytometry, transcriptomics) in the synthesis of results, which contributed to its robustness and generalizability. However, the variance in IHC protocols, such as the use of different FOXP3 antibody clones or scoring cutoffs (e.g., percentage of TILs versus stromal cells), introduced

methodological variability that accounted for a moderate statistical heterogeneity (I^2 = 68%) (Miyara & Sakaguchi, 2011). The underrepresentation of African and Oceanian cohorts serves to limit applicability to these populations. Lastly, the abundance of cross sectional designs prohibited an analysis of Treg dynamics during treatment from a temporal perspective.

Future Research Recommendations

1. Standardization of Treg protocols, including consensual guidelines for IHC staining and scoring validated by flow cytometry and single-cell RNA signatures (Li et al., 2016).
2. Prospective longitudinal studies on Treg pre-, during, and post-therapy will resolve immune remodeling and inform optimal Treg-intervention windows.
3. Complex multi-omics approaches (spatial transcriptomics, multiplex immunofluorescence) should be implemented to depict and interrogate Treg spatial and functional heterogeneity within the tumor niche.
4. Include diverse populations and conduct studies in under-represented regions and ethnic groups so that results are relevant to populations globally.
5. Embed Treg stratification within clinical trials that open the way for directly answering the question of whether Treg-high patients benefit more from Treg-modulatory therapies or combination checkpoint blockade regimens.

CONCLUSION

T_{reg} constitute a major immune-suppressive population within the breast tumor environment, with a higher incidence in aggressive and advanced disease stages. Considerable effort should be made to standardize quantification of Treg-CI, whether through harmonized IHC protocols or transcriptomic signatures validated across laboratories-into clinical practice to enhance patient prognostication and benefit from therapies targeting Treg. Future efforts should aim for longitudinal sampling, assay harmonization across laboratories, and incorporation of Treg stratification in immunotherapy trials to capitalize on such knowledge for the betterment of patient outcomes

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